This guidance was written prior to the February 27, 1997 implementation of FDA's Good Guidance Practices, GGP's. It does not create or confer rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both. This guidance will be updated in the next revision to include the standard elements of GGP's.

Review Criteria for Assessment of In Vitro Diagnostic Devices for Direct Detection of Mycobacterium SPP

February 28, 1994

REVIEW CRITERIA FOR ASSESSMENT OF IN VITRO DIAGNOSTIC DEVICES FOR DIRECT DETECTION OF MYCOBACTERIUM SPP. (DCLD/Microbiology Branch)

This document represents the current major concerns and suggestions regarding in vitro diagnostic devices employing nucleic acid amplification and hybridization, or other methodologies, for direct detection of Mycobacterium tuberculosis and/or other Mycobacterium spp. in clinical specimens. It is based on 1) current basic science, 2) clinical experience, 3) previous submissions by manufacturers to the FDA, and 4) the Safe Medical Devices Act of 1990 (SMDA) and FDA regulations in the Code of Federal Regulations (CFR). This document is intended to be flexible and as advances are made in science and medicine, these review criteria will be re-evaluated and revised as necessary to accommodate new knowledge.

PURPOSE: The purpose of this document is to provide guidance on information to present to the Food and Drug Administration (FDA) before a device to detect Mycobacterium spp. directly in clinical specimens may be cleared for marketing.

DEFINITION: This generic type device is intended for use in clinical laboratories as an in vitro diagnostic test for qualitative detection of Mycobacterium [species or group] [specific nucleic acid sequence, e.g., IS6110] directly in [type(s) of clinical specimen] by [type of nucleic acid] amplification and hybridization (or other methodology).

PRODUCT CODE(S):

REGULATION NUMBER: 21 CFR § 866.3370

CLASSIFICATION:

PANEL: MICROBIOLOGY (83)

REVIEW REQUIRED: Premarket Notification [510(k)]

I. BACKGROUND

The definitive diagnosis of tuberculosis and other mycobacterial diseases is dependent on the isolation and identification of the etiologic agent[1]. Microscopic examination, even though relatively insensitive (requiring at least 5 X 10^3 organisms per mL of specimen for detection), is the method used for screening. The number of organisms present in pulmonary secretions is directly related to the risk of transmission and 50 to 80% of patients with pulmonary tuberculosis will have positive smears[2]. Legally marketed devices for the identification (presumptive and definitive) of Mycobacterium spp. are available from various manufacturers using the following technologies:

1) Nucleic acid hybridization: this method is insensitive for direct detection of Mycobacterium spp. at less than 10^4 CFU/mL and is used primarily for culture

confirmation of Mycobacterium spp. grown on solid or liquid mycobacteriological culture media.

2) Microscopic screening for acid fast bacilli using basic fuchsin or fluorochrome stains: fluorescent microscopy using auramine-rhodamine stain is the preferred method, because it has increased sensitivity and allows more rapid examination of slides[1]. A cytocentrifugation method has also been reported to optimize sensitivity[3].

   Microscopy also is used to assess patient infectiousness because it gives a quantitative estimation of the number of bacilli being excreted.[2]

3) Conventional culture (e.g., using radiometric, selective broth with isolation and identification by NAP inhibition, nucleic acid hybridization, biochemical testing, or HPLC to detect species-specific mycolic acids): culture is able to detect as few as 10 organisms/mL of digested, concentrated clinical material[2] and is the definitive diagnosis for tuberculosis when M. tuberculosis group is identified.

4) Tuberculin skin test: this is the traditional method for demonstrating infection with M. tuberculosis. Immunocompromised individuals may be anergic and have a limited ability to respond even when infected with M. tuberculosis; therefore, a negative reaction will not rule out infection. A positive skin test does not necessarily signify the presence of disease, but will support the diagnosis of tuberculosis in patients with suggestive clinical signs, identify previously infected persons who could benefit from preventive therapy, and can be used for surveillance to identify newly infected persons[2].

Nucleic acid amplification/hybridization procedures are used in research laboratories. These methods are specific and potentially more sensitive for direct detection in clinical specimens.

Described methods using nucleic acid amplification techniques for detection of mycobacteria in clinical specimens use different signature targets (e.g., the 65-kDa heat shock protein, IS6110, MBP 70, MBP 64, a 38-kDa protein, and 16S rRNA).[4]

Only the isolation and identification of viable M. tuberculosis is definitive evidence of disease (tuberculosis). The relation between the identification of M. tuberculosis nucleic acid directly in clinical samples and the presence of clinical disease has not been firmly established. The clinical significance of the culture isolation of nontuberculous Mycobacterium spp. must be evaluated by presence of other clinical evidence (radiography, symptomatology, risk factors, immunological status, etc.).

The reemergence of tuberculosis as a major public health problem

in the United States has been widely discussed both in scientific and non-scientific publications[5,6]. Major issues in attempts to control transmission of tuberculosis are the association of tuberculosis with HIV positivity, rapid disease development and progression in immunocompromised individuals, isolations of multidrug-resistant (MDR) strains, nosocomial and institutional outbreaks, and heightened concerns for protection of health care workers. Rapid and, safe and effective diagnostic tests are needed to implement necessary infection control procedures and provide appropriate patient care.

II. DEVICE DESCRIPTION

Key issues in the 510(k) review of a new device are the specific intended use (the organism detected and the indications for use), clinical utility (how the device will be used in the clinical setting), the type of specimen tested, and the technology utilized. The following descriptive information must be included in a 510(k) submission to adequately characterize the new *in vitro* device for direct detection of *Mycobacterium* spp. in clinical specimens. Appropriate peer-reviewed literature references that relate to the technology used must be submitted.

A. Intended Use.

Describe the intended use based on the technology/methodology employed in the device. The following questions should be addressed:

1. What populations should be tested?

2. What are the conditions and limitations for use of the device when used to diagnose tuberculosis or other mycobacterial infections (active and/or latent) directly from patient specimens?

3. How will the device be used in a laboratory diagnostic algorithm? What other clinical/laboratory tests must be done?

   Examples:

   a. Presumptive determination of presence of *M. tuberculosis* group (with no determination of viability) that would be performed on all specimens; even if the device is species-specific and has a high sensitivity, all specimens would require culture for identification of dual infection with another mycobacterial species, susceptibility testing, and possibly viability determination to distinguish successfully treated from unsuccessfully treated patients. This type of device could be used in conjunction with the AFB smear or potentially with a genus-specific direct detection device for detection of

previously undiagnosed mycobacterial disease.

b. Presumptive detection of Mycobacterium spp.; the device is genus-specific and if sufficiently sensitive could be used to replace AFB smear for screening; ideally (if 100% sensitive), negative specimens would not require culture. Positive specimens would require culture to determine viability, detect multiple infections, identify species, and perform susceptibility testing.

B. Detailed Principle of the Test Methodology.

Discuss the principle of the test methodology(ies) in detail. Provide information to substantiate application of the methodology to the detection of Mycobacterium spp. Cite peer-reviewed literature references where appropriate. Describe similarities and differences in technology, procedure methodology, and design of the new device as compared to a device of similar technology and design that is legally marketed in the United States. Include a complete description of the following components as appropriate (see Review Criteria for Assessment of Nucleic Acid Amplification-based in vitro Diagnostic devices for direct Detection of Infectious Microorganisms for detailing of methodological information required):

1. Extraction/pretreatment procedure.

2. Primer(s) utilized and target (signature) sequence(s).

3. Amplification enzyme(s).

4. Nucleic acid synthesis nucleotides.

5. Detection probe(s) used for amplification products.

6. Enzymatic, fluorescent, or other substrate used to detect hybridization complexes.

7. Determination of the cut-off value(s) or endpoint(s) for the assay; this may be validated using data as described in III.A.1.

8. Controls/calibrators included in the assay kit and describe what aspects of the procedure are controlled and not controlled.

9. Any additional reagents or methods which contribute to the effectiveness of the device.

10. Safety aspects for performing the assay. Specify at what procedural step the testing material is non-infectious.

11. Collection and transport materials provided in the kit or recommended for use.

12. Software elements and dedicated instrumentation that are responsible for specimen handling, amplification, and/or detection. See requirements for Moderate Level of concern in Reviewer Guidance for Computer Controlled Medical Devices Undergoing 510(k) Review (available from the Division of Small Manufacturers Assistance).

C. Specimen collection/transport devices.

Detail the method(s) that must be used to collect optimal specimens for testing. Provide references for proper specimen collection procedures. Describe any considerations or differences in specimen transport, storage, or preparation/extraction dependent on the type of specimen.

1. Specify the types and volume (if applicable) of all specimens acceptable for testing with the device. Discuss the effects of testing inadequate or inappropriate specimens.

2. List the appropriate specimen transport conditions (e.g., time, temperature, etc.) for each type of specimen. List and discuss the effect(s) of inappropriate transport.

3. Describe recommended storage time and temperature.

D. Merits and Limitations of the Methodology(s).

Discuss the merits and limitations/advantages/disadvantages of the test methodology(ies) of the new device compared to other available test methodologies. Concerns with nucleic acid amplification methods are: adequate sample preparation, presence of sample-related or other inhibitors, risks of sample contamination, adequate quality control, relationship of a positive/negative result to disease presence/absence, and ability of laboratories to reproducibly detect a consistent level of organism load.

III. SPECIFIC PERFORMANCE CHARACTERISTICS

The FDA requests certain types and amounts of data and statistical analyses to market in-vitro diagnostic devices. The amount and types of data requested depend on the intended use and the technological characteristics of the new device. The data and statistical evaluation should be sufficient to determine if the device is safe and effective for all claimed specimen type(s). Additional data may be necessary to substantiate certain claims of intended use or clinical significance, or to validate use of a new technology.

Provide complete procedures for all studies. All testing to establish the performance characteristics of the device must be performed with the device in its final specified format according to instructions provided in the product labeling. Present test data with analyses and conclusions; include explanations for unexpected results and any additional testing performed. When appropriate, charts (scattergrams, histograms, ROC curves, etc.) may be used as part of analyses and conclusions. Raw data may be requested.

Submission of the following data is required to determine the ability to detect Mycobacterium spp. using nucleic acid amplification and hybridization methodologies:

A. Analytical Laboratory Studies (Phase I and II)

Specific parameters of importance to the operation of the device should be supported by data determined with the device prior to testing in outside laboratories. Testing should be done in-house or at a designated laboratory facility as part of the test development phase.

1. Validation of Cut-off

Describe the rationale for determination of the assay cut-off(s). Data should demonstrate that the cut-off has been appropriately selected by testing the following:

a. A minimum of 100 specimens* determined to be positive by smear and/or culture and including specimens with fewer than 10 colonies/mL from digested, concentrated material in culture.

b. A minimum of 100 specimens* determined to be negative by culture.

* Frozen specimens may be used

All false positive and false negative results should be completely analyzed; if co-amplification is not part of the device procedure, spike-back must be done to determine incidence of non-specific inhibition as a cause of false negativity; in addition, culture isolate amplification to determine if the strain is amplifiable/detectable with the device must be done for any false negatives. False positives (device positive/culture negative) may be clarified by clinical information (positive radiology, other positive cultures, prior anti-tuberculous treatment, etc.). Demonstrate that amplified products in ten of the positive specimens contain sequences of the expected size (e.g., by restriction site analysis, agarose gel electrophoresis, etc.).

2. Limits of Detection.

The analytical sensitivity (limits of detection) of the assay must be determined using known mycobacterial isolates, harvested at the exponential growth phase. Dilute fresh cultures serially, test in triplicate with the device, and compare to direct AFB smear and quantitative culture. Present data as lowest dilution of CFU or AFB per mL. Dilutional studies must be done for a minimum of 25 to 30 isolates representing geographic and phenotypic diversity, including drug susceptible, INH-resistant, and MDR strains. List source of isolates. Demonstrate that amplified products contain nucleic acid sequences with the expected size. Correlate this data with similar data based on detection of target copies.

3. Device Specificity

The specificity of the new device for amplifying and detecting a particular species or genus should be demonstrated as completely as possible. The cross-reactivity should be investigated using the assay system with sufficiently challenging concentrations (10^7 to 10^8 CFU/mL for bacterial strains). Any species that have any homology should also be tested. Strains of the following microorganisms should be tested for cross-reactivity when the device is to be used for respiratory specimens in addition to any others that would be encountered as pathogens or normal flora in the other patient specimen types that will be tested:

For M. tuberculosis-specific devices: M. bovis, M. africanum, M. microti
other mycobacterial species not detected by the device
Nocardia asteroides, N. brasiliensis, N. farcinica, N. otitidiscaviarum
Streptomyces spp.
Actinomyces spp.
Propionibacterium acnes
Aeromonas hydrophila
Blastomyces dermatitidis
Bordetella pertussis
Candida albicans
Citrobacter freundii
Corynebacterium diphtheriae
Corynebacterium spp. (e.g. C. jeikeium)
Chlamydia trachomatis, C. pneumoniae
Coccidioides immitis
Cryptococcus neoformans
Eikenella corrodens
Enterobacter aerogenes, E. cloacae
Enterococcus faecalis, E. faecium
Escherichia coli
Fusobacterium spp.

Haemophilus influenzae, parainfluenzae
Histoplasma capsulatum
Klebsiella pneumoniae, K. ozaenae
Lactobacillus spp.
Legionella pneumophila, L. micdadei
Moraxella catarrhalis
Mycoplasma pneumoniae
Neisseria meningitidis, N. gonorrhoeae
non-hemolytic streptococci
Peptostreptococcus spp.
Porphyromonas spp.
Prevotella spp.
Pseudomonas aeruginosa
Salmonella enteritidis, Salmonella typhi
Staphylococcus aureus (include protein A-producing)
Staphylococcus epidermidis
Streptococcus pyogenes and other hemolytic streptococci
Serratia marcescens
Streptococcus pneumoniae
Veillonella spp.
Xanthomonas maltophilia

Viruses: respiratory syncytial virus, adenovirus, cytomegalovirus, enterovirus, herpesvirus, influenza, parainfluenza, rhinovirus

4. Interference Studies.

Any potentially cross-reacting or interfering substances that may be encountered in specific specimen types, or conditions should be tested using the assay system with target organism close to the limit of detection.

This would include but not be limited to blood, mucus, human leukocytes, nebulizing solutions, anesthetics and/or other exogenous materials used in the specimen collection process or potentially encountered in patient specimens. Additionally, freeze-thaw cycles, anticipated adverse transport and storage conditions must also be investigated.

Verify that recommended specimen storage and transport conditions are compatible with the assay. State the optimal conditions based on real-time specimen storage stability studies. Both false positivity and false negativity should be evaluated.

5. Precision

The National Committee for Clinical Laboratory Standards (NCCLS) recommends an analysis of variance experiment that permits estimation of within-run and total standard deviations (SD).[7] See EP5-T2 (NCCLS Guideline) for recommended data collection formats and calculations. Perform separate calculations for each specimen tested

for within-run and total precision.

For all test formats, test a minimum of two negative, two low positive (less than 50 CFU), and two moderately high positives (100-1000 CFU) in addition to controls included in the assay kit, three times in each of two runs on three different days. Specimens may be spiked specimens prepared by adding the organisms to pooled negative specimens.

Precision studies should also be done on the same specimens at the two outside laboratories performing comparative studies, in addition to the manufacturer's laboratory. Controls contained in the test kit should also be included.

For calculated endpoint tests, present coefficients of variation for each set of values for with-in run and total precision, using absorbance values and reporting units defined in the test procedure.

For single endpoint assays, provide percentage of results negative, borderline/equivocal, or positive for each set of tests.

If dedicated instrumentation is used in specimen handling, or reading and interpreting results, use a different instrument at each site. If non-dedicated instruments are used, state specifications of instrument(s) used at each site.

B. Clinical Studies.

Comparison studies provide data on the ability of the system to accurately detect Mycobacterium spp. It should be demonstrated that the performance of the device is substantially equivalent to culture and/or fluorochrome-stained smears (depending on intended use) by comparison to a well-controlled culture method at a minimum of three different clinical sites representing diverse geographic regions and diverse patient populations (e.g., New York City, California, Texas, and/or Florida; HIV-positive, IV drug abusers, low socioeconomic status groups, etc.). Provide the names and telephone numbers of principal investigators and sites at which testing was performed. The laboratories must not be affiliated with the manufacturer and must perform at least level III mycobacteriology procedures.

Justify the choice of methods used and include references. Procedure protocols must be included in the submission, including methods used for grading smears and assessing culture semi-quantitation, specimen collection methods, transit time between collection and culture, storage conditions prior to culture, and specific laboratory culture procedures, including quality control procedures performed

and methods of quantitation. Initial screening of AFB smears should be done using auramine-rhodamine stain (or another method verified by control procedures to be capable of consistently detecting 10^3 AFB/mL. Include in the data presentation the relative numbers of organisms cultured, AFB smear semi-quantitation, and species identity of all mycobacterial isolates.

The new device under evaluation must be used in conformity with procedures and recommendations specified in the product insert. All borderline/equivocal results should be repeated. Initial new device results may not be changed (resolved) for the final analysis. Culture amplification to determine if the strain is amplifiable/detectable with the device may be done retrospectively after results are unblinded. False positives (device positive/culture negative) may be clarified by clinical information (positive radiology, other positive cultures, prior anti-tuberculous treatment, exposure history and skin test conversion, etc.) for final data analysis.

Perform the tests on an adequate number of positive and negative clinical specimens (following collection, storage, and testing instructions recommended in the package inserts). The 95% confidence limits of sensitivity and specificity point estimates must be sufficiently narrow (e.g., plus or minus 5%) to evaluate clinical utility.

1. Describe the clinical protocol design in detail and consider the following:

    a. All testing must use fresh clinical specimens (e.g., expectorated and induced sputa, endotracheal and bronchial aspirates, bronchoalveolar lavages, etc.) routinely received in the laboratory for AFB smear and culture, and must be processed concurrently for AFB smear, culture and the new test.

    b. Specimens with other Mycobacterium spp. isolated (e.g., M. gordonae, M. avium-intracellulare, etc.) must be represented.

    c. Document patient history (including previous history of tuberculosis): current treatment for tuberculosis at time specimen is taken; number of specimens submitted for smear/culture; PPD results; presence or absence of clinical evidence of active disease; final clinical diagnosis; and radiographic findings.

    d. Document laboratory findings for each specimen and include graded smear results, semi-quantitative culture results, and specimen type (expectorated, induced, lavage, etc.). Provide identification (to species level) of all mycobacterial species

isolated.

e. Provide the study protocol used at each site. This should indicate appropriate safety precautions required, specimen selection criteria, specimen storage and handling procedures, culture and smear methodologies, identification procedures, blinding procedures, and how results are documented, reviewed and analyzed. All test parameters must be established prior to initiation of the clinical studies. Document all quality control results and repeat tests for runs with out-of-range QC values.

f. Document for each site the criteria for specimen inclusion in the study. Selection of test specimens must be unbiased and testing must be performed in accordance with routine laboratory practices. The new assay must be used during the study in accordance with its intended use and specimens tested must represent appropriate populations for the disease (patients with symptoms suggestive of tuberculosis, persons with history of exposure or skin test converters, etc.) and include appropriate groups in which tuberculosis is commonly found today (e.g., HIV positive, IV drug abusers, etc.).

2. Include results of testing a minimum of 100 sputum specimens obtained from patients with other pulmonary diseases (fungal, viral, or bacterial infections). Provide final clinical diagnosis for these patients and skin test results, along with laboratory test results (AFB culture, smear and new device) as a data subset.

3. Each site should test a minimum of number of specimens to obtain a minimum of 20 positive patients (the majority of patients will have 2 to 5 specimens submitted); overall, minimally 150 positive patients (minimally 300 total positive specimens) should be represented to achieve acceptable confidence limits for an overall positivity rate of 10%.

4. If more than one specimen type (i.e., sources other than digested/decontaminated and concentrated respiratory specimens) is indicated for testing with the new device, provide additional data to include minimally 20 total positive patient specimens from each additional source.

5. Categorize the final data analysis by site, patient diagnosis (active disease, pulmonary and extra-pulmonary, if appropriate; treated disease; reactivated disease; skin test positives or converters with no clinical symptoms of disease), and HIV status.

C. Reproducibility

Provide results of an interlaboratory comparison of testing of at least 25 specimens by three laboratories different than those performing the Clinical Studies (not necessarily Level III laboratories). Specimens may be lyophilized, frozen, or fresh transported according to labeling instructions. Specimens should include a diversity of organism concentrations (negative, less than 10/mL, 10 to 50/mL, 50 to 100/mL, etc.). All should be handled as specified in the labeling instructions. Specimens should be coded and results should be assessed by a single designated contact. Negative controls should be run between test samples to test for sample carry-over.

IV. LABELING CONSIDERATIONS

The following are additional details for some of the points in the statute [502(f)(1)] and regulations [21 CFR § 809.10(b)].

A. The Intended Use Statement

The intended use statement should be a concise description of the essential information about the product. It should communicate the following information:

1. Test methodology, including the nucleic acid sequence(s) that are amplified and detected.

2. Whether the assay specifically detects M. tuberculosis or another species, or whether the assay detects all or multiple mycobacterial species.

3. What specimen sources may be tested.

4. If the assay is to be used only with a special instrument.

   Example:

   XXX is for the detection of Mycobacterium [species or group] [name specific nucleic acid sequence, e.g., IS6110] directly in [state type(s) of clinical specimen] by [specify type of nucleic acid] amplification and hybridization (or specify other methodology).

B. Conditions for Use

Conditions for use of the device should describe any special applications of the device or specific contraindications or indications for use that are not addressed in the Intended Use Statement, e.g., "for digested, decontaminated and concentrated respiratory secretions as an adjunct to mycobacterial culture. This test may only be performed using

Biosafety Level 2 practices and a Class I or II biological safety cabinet."

These conditions for use may be addressed further in either the Summary and Explanation, Limitations, or Performance Characteristics Section of the package insert. Safety instructions must be included in the Warnings and Precautions section and be incorporated into the test procedural instructions.

C. Specimen Collection and Handling

1. State the type of specimen to be collected, and the types of collection devices which may be used.

2. State the conditions for patient preparation, e.g., timing of collection, order of collection, etc.

3. Provide adequate directions for sample collection and/or references for appropriate collection procedures, e.g., textbooks, journals, etc.

4. Identify interfering substances or conditions.

5. Provide instructions for transport to the laboratory for testing.

6. State the specimen storage conditions and stability periods.

7. Provide a recommendations for the laboratory to assure collection and transport requirements have been followed.

D. Quality Control

Information provided in a Quality Control section should include the following:

1. State which ATCC organisms or commercially available products should be used for positive and negative quality control, if materials are not provided in the kit. Advise user if controls that are provided challenge all aspects of the procedure and recommend alternative control measures to challenge those steps not covered.

2. Provide recommendations for frequency of quality control and placement of controls and samples in the assay.

3. Provide directions for interpretation of the results of quality control samples.

4. The Quality Control section should conclude with a statement similar to the following: "If controls do not

behave as expected, results are invalid and patient results should not be reported".

E. Expected Results

1. Provide expected prevalence of mycobacterial infections in different populations and from appropriate specimen sites using the new device.

2. Indicate that prevalence may vary depending on geographical location, age, sex of population studied, type of test employed, clinical and epidemiological history of individual patients, etc.

F. Limitations of the Test.

List important test limitations and all known contraindications, with references when appropriate. All tests which claim to detect mycobacterial species must include at least the following in the Limitations Section, as appropriate for specimen types, unless performance data or corroborated references have been submitted to substantiate that a particular limitation or contraindication does not apply.

1. Test only indicated specimen types. Testing of other specimens types may result in false negative or positive results.

2. The predictive value of a positive test decreases when prevalence decreases. Interpretation of positive results in a low risk patient population should be made with caution. Usefulness of this test has only been established in testing [specimen types] in [types of populations].

3. Reliable results are dependent on adequate specimen collection and proper transport procedures.

4. Therapeutic failure or success cannot be determined as mycobacterial nucleic acid may be detected following appropriate chemotherapy.

5. Interference by endogenous and exogenous substances has not been established.

6. For those assays using a genus-specific technology, a statement indicating that the assay will not specifically differentiate M. tuberculosis from other acid fast bacilli.

7. Detection of mycobacterial species is dependent on the number of organisms present in specimens. This may be affected by specimen collection methods and patient factors such as age, presence of symptoms, prior

treatment, etc.

8. The minimum detection level of this test may vary according to mycobacterial species present.

G. Performance Characteristics:

Provide summaries of the performance data for the assay, e.g., clinical sensitivity and specificity compared to culture; and clinical sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for detecting active disease. Positive and negative predictive values should be based on specific populations sampled for each specimen type. State the prevalence at each testing site. Include 95% confidence intervals.

1. Present cross-reactivity studies in a tabular form; specify all positive and borderline/equivocal/indeterminate results.

2. Present limits of detection for all species or all strain variants as appropriate. Include data both for number of copies and number of organisms (CFU) detected.

3. Summarize within-run and total precision.

4. Summarize reproducibility data.

5. Present data from clinical studies, using separate categories for different patient groups and specimen sources. Clearly display all borderline/equivocal/indeterminate results. Discrepancies between test and culture results (or clinical diagnosis) may be discussed. Present final sensitivity, specificity, positive and negative predictive values, based on the total number of true positive and negative specimens determined by culture results, clinical diagnosis based on other clinical/-laboratory information, or a combination. Define methods for determining true positives and negatives. Include 95% confidence limits along with point estimates of the above parameters.

V. BIBLIOGRAPHY.

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4. Kirschner, A Meier, EC Bottger. Genotypic identification and detection of mycobacteria-facing novel and uncultured pathogens, p173-190. In DH Persing, TF Smith, FC Tenover and TJ White (ed), Diagnostic Molecular Microbiology, Principles and Applications. American Society for Microbiology, 1993, Washington DC.

5. Bloom, BR, CJ Murray. 1992. Tuberculosis: commentary of a reemergent killer. Science 257:1055-64.

6. Cowley G, EA Leonard. Tuberculosis: an old killer returns. Newsweek, March 16, 1992; 53-56.

7. National Committee for Clinical Laboratory Standards. Evaluation of precision performance of clinical chemistry devices, tentative guideline. 1991. Order code EP5-T2.

NOTE TO: Interested Manufacturers

DATE: March 8, 1994

FROM: Chief, Microbiology Branch, Division of Clinical Laboratory Devices, Center for Devices and Radiological Health

SUBJECT: Review Criteria for Assessment of In Vitro Diagnostic Devices for Direct Detection of Mycobacterium spp.

Since we developed the draft document entitled, "Assessment of In Vitro Diagnostic Devices for Direct Detection of Mycobacterium spp.," we have received questions about specific issues in the "Clinical Studies" section. In order to clarify some of these issues and provide more assistance in directing clinical studies protocols, we have prepared attachments to the original guidance document. These attachments are also available from the Division of Small Manufacturers Assistance (DSMA), telephone 800-638-2041.

Since this area of in vitro diagnostics is rapidly expanding in the clinical laboratory, we are soliciting your ideas, recommendations, and comments regarding the attachments to the review criteria. We will appreciate receiving your comments so that we can incorporate as many improvements as possible in a revision.

Please address comments to:

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Attachments

ATTACHMENT 1

REVIEW CRITERIA FOR ASSESSMENT OF IN VITRO DIAGNOSTIC DEVICES FOR DIRECT DETECTION OF MYCOBACTERIUM SPP. (DCLD/Microbiology Branch)

FDA includes the following additional information to assist sponsors in the collection of data and information that will be used to assess the safety and effectiveness of a new device for the detection of Mycobacterium tuberculosis:

1. **Sample Clinical Data Form** (Attachment 2)

   This form outlines the minimal clinical information that may be used to evaluate the clinical sensitivity and specificity of M. tuberculosis detection devices. In addition to demographic information, the following information should be documented in order to assess patient clinical category:

   a. **Past history** of TB including when the patient was diagnosed and whether treatment was effective; whether a previous PPD was positive; the patient's exposure history.

   b. **Current clinical evaluation** to assess patient diagnosis. This clinical documentation must often be obtained prospectively from patient charts after cultures are ordered.

      (1) PPD done during the **current** clinical presentation.

      (2) Radiographic results

      (3) Current bacteriologic testing

   c. The **current clinical diagnosis or suspected diagnosis** [ATS (American Thoracic Society) patient categorization].

      Although the diagnosis, treatment and control of tuberculosis is evolving with new technology and with increased understanding of pathogenesis in different patient populations, the ATS-CDC recommendations described in "Diagnostic Standards and Classification of Tuberculosis" (Am Rev Respir Dis, 1990; 142:725-735) for classification of persons exposed to and/or infected with M. tuberculosis is appropriate to use for patient categorization. In order to establish the safety and effectiveness of any device for direct detection of Mtb, the patient population for whom the test is useful must

be defined.

For a device that detects M. tuberculosis directly in patient specimens, the clinical utility is to detect new cases of tuberculosis in order to initiate appropriate chemotherapy and isolate patients in order to control further transmission. Testing large numbers of patients who have already been diagnosed with tuberculosis will not establish the safety and effectiveness of the device for patients with suspected tuberculosis who present the greatest public health concern. For this reason, FDA will evaluate not only a comparison to culture but also the clinical sensitivity and specificity of the device for the initial diagnosis of tuberculosis in previously undiagnosed patients (ATS Class 5). Testing of large numbers of patients in Class 3 (clinically active tuberculosis) may not be useful for establishing the clinical utility of prospectively diagnosing new cases of tuberculosis. Additional types of clinical data would be needed to establish any claims for monitoring therapeutic effectiveness and/or completeness. The use of any device in ATS Class 3 patients would involve analysis of different types of data (e.g., serial specimens for monitoring response to therapy). It is important that these patient populations are distinguished for the clinical studies to have the appropriate significance.

Review of patient charts will be necessary to determine patient classification. It is important to distinguish the initial categorization from final diagnosis which will be determined in most cases by culture/smear results. However, up to 20% or more of patients with tuberculosis may be culture negative. Determination of the correct classification diagnosis for these patients is dependent on clinical and radiographic findings showing either stable or improving chest radiographs.

Therefore, patient followup will be important to assess the clinical significance of [any new test positive/culture negative] patients and also [new test negative/culture negative] persons who are Class 5 and receiving chemotherapy (empiric therapeutic or prophylactic). Culture negativity would include those with single or multiple negative specimens. Any patient with any positive culture during the current clinical course is by definition recategorized into ATS Class 3.

All Class 5 patients should be clinically recategorized within 3 months unless lost to followup. The FDA would

consider an expected 20% lost to followup group normal in the usual patient populations involved in tuberculosis clinical studies.

2. **Sample Laboratory Data Form** (Attachment 3)

This form outlines the types of information that FDA believes will be important in assuring the safety and effectiveness of new devices for routine laboratory use and for determining the appropriate labeling (package insert specimen handling and procedural instructions and quality control parameters).

a. **Specimen collection and handling** information: it is imperative to establish optimal specimen handling procedures prior to initiation of clinical studies. Since this aspect of the study is often the most difficult to control and may affect the results obtained, it is necessary to document the transport and storage conditions of the specimen.

b. All relevant information pertaining to **direct microscopic and culture results** should be documented. Both direct smear and culture results should be semiquantitated. Although the Bactec system is well-established as a rapid and effective method to detect mycobacteria (usually in conjunction with specific probes), there is no accepted method of quantitation; sponsors are encouraged to include a solid media in the culture protocol. The clinical protocol must provide all specific information for the procedures used for culture and direct microscopic testing (e.g., concentration procedure, inoculation procedure, stain and reporting format, media and incubation conditions, method of identification, quality control procedures, etc.).

c. All relevant information pertaining to the results for the new device must be documented. The procedures used for processing and testing must adhere to the protocol; if changes are made during the course of the study, they must be completely documented and validated with appropriate supporting data, and results analyzed separately along with justification for pooling with other data sets. All quality control testing must be documented for each run/plate and should include positive (fewer than 50 copies) and multiple negative controls. Intrinsic controls and spikebacks for negative samples are encouraged. Instruments (e.g., thermocyclers) used should be validated for reproducibility and accuracy of performance (e.g.,

temperature and timing cycles). Include a printout of the cycling program and/or a representative run printout in the 510(k) submission. Include results of any other testing procedures done on each specimen (e.g., repeat testing, alternate primer pair testing, etc.).

d. In addition to straight comparison to culture, FDA will require comparison to clinical diagnosis for culture negative presumptively treated patients, which would be included in the final data analysis. Each sponsor should develop appropriate spreadsheets/data entry procedures; FDA will routinely request line data for review.

e. Each clinical investigator must verify that the data forms represent the testing done in the investigating laboratory.

Sample Laboratory Data Form
Direct Tests for *M. tuberculosis*

Specimen

1. PATIENT IDENTIFIER:_____ 2. Date Collected:_____

3. Source:
   - Sputum
     - Expectorated
     - Induced
   - BAL
   - Other _____

4. Volume:_____mL

5. Appearance:
   - Bloody
   - Purulent
   - Watery

6. Date/Time Received in lab:_____ 7. Transport Time:_____

8. Storage Temp: rm temp/2-4•/-20•/-70•

Culture/Smear

8. Date Processed:_____

9. Direct Smear: No AFB seen 1+ 2+ 3+ 4+

10. Date Culture Positive:_____ No AFB @ 6/8 wk

11. Identification:_____ GLC Biochemical Probe

Amplification Test

12 Date Tested: _____ 13. Run/Plate*:_____

14. Result:_____ 15. Interpretation: Positive Negative Equivocal

16. Repeat Reason:_____

Date:_____ Run/Plate_____ Result:_____

* QC results for each run/plate are recorded on QC worksheet

17. Other tests done (may include spike-back, co-amplification, amplification of alternate primer pair, etc.)

Comparison to Culture: TP FP TN FN

Institution:_____

Information reviewed by:

_____

Signature Date

DCLD Final Draft:3/7/94
Attachement 3, Mycobacteria Guidance Document

Sample Clinical Data Form
Direct Tests for *M. tuberculosis*

Demographic Data

1. **PATIENT IDENTIFIER** 2. Date of Birth: 3. Sex: Male Female

4. Race:
White
Black
American Indian/Alaskan Native
Asian/Pacific Islander
Other _____
Unknown

5. Foreign-born: Yes No
Years in U.S. _____
Country of birth _____

Past Clinical History

6. **Past History of TB:** Yes No

Month/Year _____ Type: Pulmonary Extra-pulmonary

Treatment Date started: _____ Date ended: _____

Treatment effective: Yes No

7. **Previous PPD:** Positive Negative Date: _____

8. **Exposure:** more than 3 months ago within past 3 months

Current Clinical History

9. **Inpatient/Outpatient** Admission Date: _____ Discharge Date: _____

10. **HIV status:** positive negative

11. **CLINICAL EVALUATION** Date: _____

Signs & Symptoms: fever weight loss night sweats hemoptysis

malaise cough other _____

12. **PPD** Date _____ _____mm induration

positive negative

13. **Radiography (Chest X-ray)** Date: _____

normal cavitary noncavitary infiltrate

miliary pattern other pattern: _____

stable unstable (improving/worsening)

14. **Bacteriology**

Specimen Date	Source	Gram Stain	AFB Smear	Culture ID

Initial Clinical Diagnosis (See classification in Diagnostic Standards and Classification of tuberculosis; Am Rev Respir Dis, 1990; 142:725-735)

0 No exposure/not infected

1 Exposure history/no evidence of infection

2 TB infection/ no evidence of disease

3. Clinically active TB:

   a. Under treatment

   b. Not treated

4. TB/not clinically active

5. TB suspected

   a. Under treatment . . . . . .3 MONTH FOLLOWUP DIAGNOSIS: _____

   b. Not treated

Final Diagnosis

Clinically Active TB No Clinically Active TB

Institution:_____

Clinical Data completed by:_____ _____

Print Name Signature Date

Information reviewed by Principle Investigator:

_____

Signature Date